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Research Paper

Chemical composition and biological activities of *Ficus capensis* leaves extracts

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ABSTRACT

The leaves of *Ficus capensis* were investigated for the phytochemicals, antioxidant, anti-inflammation and antibacterial properties. Three samples were evaluated; E.O (essential oils) MWE (methanol – waters extract) and WE (water - extract). The contents of bioactive components were quantified and identified by GC/ MS, UV - spectrophotometer and RP-HPLC. The antioxidant capacities were measured using ABTS and DPPH assay expressed as IC_{50} (µg/ml).The anti - inflammation properties was quantified by the NO inhibition and the antimicrobial by the disk diffusion method. The major compounds identify in E.O are carvacrol (65.78%), α- caryophyllene (29.81), caryophyllene oxide (25.70 %), linalool (3.97%), 3-tetradecanone (2.90 %), geranylacetone (1.20), 3,7,11-trimethyl-3-hydroxy-6;10-dodecadiene-1-yl acetate (1.53 %), hexahydrofarnesyl acetone (1.21 %), α-caryophyllene (0.81 %), 2-methyl-3-hexyne (0.69 %) and scytalone (0.69%). The total phenolics, total flavonoids, total anthocyanins and condensed tannins evaluate were (16.47mg gallic acid equivalent (GAE), 6.92 mg catechin equivalent (CE), 9.52 mg cyaniding glucosyl equivalent (CgE), and 4.21 mg catechin equivalent (CE) / gm dw (dry weight) of plant material respectively. Quercetin dihydrat (4.48 mg/ml) and protocathechuic acid (1.46 mg/ ml) were the major compounds identify. The IC_{50} value was 10.25 µg/ ml. The results show that these extracts have good antioxidant and antiinflammation properties. The anti microbial analyses suggest that *F. capensis* can be used in treatment of diseases caused by *E. coli* and *Bacillus subtillis.*

Keywords: *Ficus capensis*; Antioxidant; Polyphenolics; RP-HPLC; Antimicrobial.

INTRODUCTION

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products and a renewed interest has occurred in the last decade to search for phytochemicals of native and naturalized plants for pharmaceutical and nutritional purposes (Oktay, et al., 2003; Wangensteen, et al., 2004). Also, it is well known that plants contain essential oils and various extracts that

may be used as alternative remedies for many infectious diseases. The antimicrobial compounds found in plants are interest because of antibiotic resistance which is becoming a worldwide public health concern especially in terms of food, borne, illness and nosocomial infections (Mora, et al., 2005; Navon-Venezia, et al., 2005). Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydroxytoluene (BHT) and butylated hydroxanisole (BHA) that are under scrutiny as suspected cancer causing agents (Wangensteen, et al., 2004; Bergfeld, et al., 2005). Plants produce a multitude of organic compounds that have anti - inflammation and antimicrobial activities. *Ficus capensis* Thumb (family-*Moraceae*) is an evergreen tree widely distributed in tropics, both leaves and roots have been used for leprosy, leucodermia and swollen fects (Nguyi, 1988; Dafalla, 2005; Oyeleke, et al., 2008). A search of the literature did not reveal any report on antioxidant and anti inflammation properties of the essential oil and the leaves extract of *F. capensis*.

The objectives of this study are to determine the phytochemicals compounds, to evaluate the antioxidant, the anti - inflammation and the antimicrobial proprieties of various samples prepared from the leaves of *Ficus capensis.*

MATERIALS AND METHODS

Dried leaves of *Ficus capensis* (Moreaceae) were obtained from Mali, and the botanical identification was carried out by Professor Max Henry, from Botanic and Mycology Laboratory, France. A Voucher specimen has been kept in our Laboratory for future reference. All plant materials were dried at room temperature and powered and sifted in a sieve (0.750 µm).

***Chemicals*:** All the chemicals used were analytical grade. 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) (ABTS), 2,2'-azo-bis(2-amidino-propane) dihydrochloride (AAPH), gallic acid, folin-Ciocalteu's phenol reagent, aluminium chloride, catechin, *p*-coumaric acid, rutin, procatechiuc acid, vitamin C, caffeic acid, isovitexin, vitexin, chlorogenic acid, catechin, quercetin, quercetin dihydrat, quercetin-3-β-D glucosyl, epicatechin, Kuromanin chloride, cyaniding chloride were purchased from Across organics (Geel, Belgium). Sodium carbonate, sodium nitrite, chlorhydric acid, ethyl acetate sodium, sodium sulphate anhydrous, ammonium phosphate, acetonitrile, methanol, interferon-gamma (IFN-γ), polysaccharide of salmonella typhimurium (LPS), sulfanilamide, NEDA (N-(1-naphtyl) ethylenediamine dihydroxy chloride), vanillin reagent, n-hexane were obtained from Sigma and Roth (Strasbourg, France). Agar nutrient (Mueller- Hinton - agar) (Becton Dickinson, Heidelberg), paper discs, ampicillin, gentamicin, nystatin, pures cultures of the bacteria (*Staphylococcus aureus* (ATCC29213), *Pseudomonas aeruginosa* (ATCC27853), *Escherichia coli* (ATCC25922), *Baccilus subtillis* (ATCC6059) and fungi *(Aspergillus niger* (135550/ 99), *Candida albicans* (ATCC90028)) were given by the I.B.I.S.E laboratory (UIT Thionville, France).

Preparation of essential oil: The essential oil was prepared according to a previously reported method (El-Massy, et al., 2009). Dried powered plant materials (250gm) were placed in a 2L round-bottom flask and mixed with 1L of deionised water. The solution was distilled for 5 h. The essential oil obtained was dried over anhydrous sodium sulphate, which was subsequently removed with filtration.

Preparation of total phenolic compounds (TPC), total flavonoid compounds (TFC) and total anthocyanin compounds (TAC) extract: Total phenolic compounds (TPC), total flavonoid compounds (TFC), and total anthocyanin compounds (TAC) were

extracted from the powders as described by Chitindingue, et al. (2007). Two grams of powdered sample were extracted twice with 10 ml of cold aqueous methanol solution (50%).

The two volumes were combined, made up to 40 ml, centrifuged at 1532 × g for 20 minutes and transferred in small sample bottles and stoked at +4 °C in the dark until analysis.

Preparation of condensed tannins (CT) extract: Samples for CT were extracted from the powders as described by Villareal-Lozoya, et al., (2007) slightly modified. 1gm of powder was extracted twice with 20 ml of n-hexane while 20 minutes, filtered and the remaining powder was dried at 35 °C under vacuum for 2 h. The powder was stored at + 4 °C until analyses.

Extraction of polyphenol compounds for RP-HPLC analysis: Polyphenols were extracted according to a slightly modified method previously described by Sharma, et al., (2005). A fresh sample (0.4gm) was extracted with 2 x 5 ml of aqueous methanol (50/ 50: v/v) with intermittent shaking (2 minutes) on vortex mixer for 30 minutes. The sample was the centrifuged at 1536 × g for 20 minutes at 20°C. The supernatant was taken into a 10 ml volumetric flask. The extract is stable for 24 h if stored at 4 °C.

Analysis of essential oils: The essential oils were analysed and identified by GC/ MS. An HP model 6890 GC equipped with a 30 m × 0.25 mm i.d. (d_f: 0.25 µm) DB-5 boded-phase fused-silica capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) were used. Injector and temperatures were 200 and 300 °C, respectively. The oven temperature was programmed from 35 to 250°C at 5°C/ min and held for 50 min. The linear velocity of the helium carrier gas was 30 cm/s. Injections were in the split-less mode. An HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/ MS) was used for mass spectral identification of the GC components at a MS ionization voltage of 70 eV. A 30 m × 0.25 mm i.d. (df = 0.25 µm) DB wax bonded-phase fused-silica capillary column (Agilent, Folsom, CA) was used for GC. The linear velocity of the helium carrier gas was 30 cm/s.

The temperature of injector and detector was 250 °C. The oven temperature was programmed from 50° to 250 at 5°C/ min and held for 50 min. The percentage of each compound in the oil is determined from peak areas without correction factors account assuming that all components have coefficients of neighboring rethinking. Identification of constituents was performed by coupling an HP model 6890 GC gas to a mass spectrometer type an HP 5791A mass selective detector (GC/MS). The volume injected is 1 ul of a pure oil solution diluted to 1% in dichloromethane. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley GC/MS Library and Nist, Tutore Libraries. The percentage composition was computed from the GC peak areas.

Dosage of phenolic compounds

Determination of total phenolic compounds (TPC): The Folin-Ciocalteu method was used to measure the total phenolic compounds (Dzingira, et al., 2007). To a sample (100 µl), distilled water was added to make 2 ml (Eppendorff tube), followed by 1 ml of Folin Ciocalteu reagent 1N and sodium carbonate (20 %). After 40 minutes at room temperature, absorbance at 725 nm was read on a Spectrophotometer against a blank that contained methanol instead to sample. The results were compared to a gallic acid calibration curve, and the total phenolic compounds were determined as gallic acid equivalents (GAE). Determination of each sample was performed in triplicate.

Determination of total flavonoid compounds (TFC): The flavonoids contents were measured according to a colorimetric assay (Kim, et al., 2003). A 250 µl of standard

solution of catechin at different concentrations or appropriately diluted samples was added to 10 ml volumetric flask containing 1 ml of distillate waters. At zero time, 75 µl of $NaNO_2$ (5%) was added to the flask. After 5 minutes, 75 µl of $AlCl_3$ (10%) was added. At 6 minutes, 500 µl of NaOH (1N) was added to mixture. Immediately, the solution was diluted by adding 2.5 distillate water and mixed thoroughly. Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. Total flavonoid compounds in medicinal plant were expressed as mg catechin equivalents (CE /g dry weight (dW). Samples were analysed in three replications.

Evaluation of total anthocyanin compounds (TAC): The total anthocyanin compound of the samples was estimated using a UV-spectrophotometer by the pH-differential method reported by Abu Bakar, et al. (2009). Two buffer systems, potassium chloride buffer, pH 1.0 (0.0025M) and sodium acetate buffer, pH 4.5 (0.4 M) were used. Briefly, 400 µl of extract was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm. Absorbance (ΔA) was calculated as: ΔA= (A510 - A700) pH1.0 - (A510 - A700) pH4.0 Monomeric anthocyanin pigment concentration in the extract was calculated and expressed as equivalent cyaniding-3 glycoside (mg l^{-1}):

$$\mathbf{\Delta A \times MW \times Df \times 1000 / (Ma \times 1)}$$

- With ΔA: difference of absorbance,
- Mw is a molecular weight for cyaniding-3-glucoside (449.2) and
- Df is the dilution factor of the samples, Ma is the a the molar absorptivity of cyaniding-3-glucoside (26.900)

Results were expressed as mg of cyaniding-3-glucoside equivalents in 100g of dried sample.

Determination of condensed tannin (CT): The condensed tannin content was estimate using the method slightly modified described by Villareal-Lozoya, et al. (2007). Briefly, an aliquot of 0.5 g of powder obtained after lixiviation (n-hexane) was placed in a centrifuge tube and 15 ml of 1% HCl in methanol was added to each sample. Each tube was vortexed and placed in a water bath at 35 °C with constant shaking for 20 minutes and vortexing every 5 minutes. After incubation, the tubes were centrifuged (1532 × g) and the supernatants were extracted. Aliquots of the supernatants (100 µl) were placed in two separate assay tubes, one for the sample determination and the other for blank determination. Samples and blanks were incubated for exactly 20 minutes after adding 5 ml of vanillin reagent (0.5 gm of reagent and 200 ml of 4% HCl methanol) to samples and 4 % HCl in methanol to the blanks. After 20 minutes, absorbance was read at 500 nm from of each sample and blank using UV-spectrophotometer Varian Cary 50.Samples absorbance was rectified with the blank standard and compared against a standard curve made with catechin. Results were expressed as mg catechin equivalent /gm of lixiviating sample (mg CE/gm). The analysis of each sample was performed at least in three replications.

Identification of polyphenolic compounds

The identification was done by RP-HPLC according to a modified method described by Sharma, et al. (2005). Extracted samples (Methanol, Methanol - water, and H_2Odd (didistillate water) were filtered through a 0.45 µm PTFE syringe tip filter, using a 20 µl sample loop. The sample was analyzed using an RP - HPLC system equipped with a waters UV-Visible tuneable detector on a reverse phase (RP C_{18}) column Alltech Interstsil ODS-5µm x 4.6 mm x 150 mm. The flow rate was set at 1 ml / minute at room temperature. To perform this study a gradient of three mobile phases was used. Solvent A: 50 mM ammonium phosphate ($NH_4H_2PO_4$) pH 2.6 (adjusted with phosphoric acid), solvent B: (80: 20 (v/v)) acetonitrile / solvent A, and solvent C: 200 mM of phosphoric acid pH 1.5 (pH adjusted with ammonium hydroxide). The

solvents were filtered through a Whatman Maidstone England paper N° 3 and putted in an ultrasonic apparatus for 25 minutes. The gradient profile was linearly change as follows (total 60 minutes): 100 % solvent A at zero minutes, 92% A / 8% B at 4 minutes, 14 % B / 86% C at 10 minutes, 16% B / 84% C at 22.5 minutes, 25 %B / 75% C at 27.5 minutes, 80 % B / 20% C at 50 minutes, 100% solvent A at 55 minutes, 100 % A at 60 minutes. After each run, the system was reconditioned for 10 minutes before analysis of next sample. Under these conditions, 20 µl of sample were injected. External polyphenolic standards were prepared by dissolving 2 mg ml^{-1} and used as external reference. Individual polyphenol in the sample were identified by comparison of their retention times with spiked in put of the corresponding external polyphenolic standards. The detection was carried out at 280 and 320 nm and their quantification was obtained by the comparison of the peaks area of individual polyphenol with the corresponding external standards peaks. All sample analysis was done in triplicate.

Antioxidant activity analysis

Two tests were used to determine the total antioxidant capacity, the DPPH and the ABTS tests.

Evaluation of radical scavenging activity by DPPH assay: The DPPH radical scavenging activity was evaluated according to a previously method slightly modified described by Pothitirat, et al. (2009). 1 ml of 100 µM DPPH solution in methanol was mixed with 1 ml of plant extract. The reaction mixture was incubated in the dark for 20 minutes and there after the optical density was recorded at 517 nm against the blank. For the control, 1 ml of DPPH solution in methanol was mixed with 1 ml of methanol and optical density of solution was recorded after 20 minutes. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity as percentage of inhibition (% IP) of DPPH radical:

$$\% \text{ IP} = [(At_0 - At_{20}) / (At_0 \times 100)]$$

- Where At_0: absorbance of sample test after zero minutes and
- At_{20}: absorbance of control after 20 minutes.
- Each assay was carried out in triplicate.

From a plot of concentration against % IP, a linear regression analysis was performed to determine the IC_{50} value for each extract. The DPPH radical scavenging activity of phenolic compounds was expressed as IC_{50} value in micrograms per ml of fresh weight. A low IC_{50} value represents a high antioxidant activity.

Determination of radical scavenging activity by DPPH assay: To 2.90 ml of an aqueous methanol solution (50%) of 100 µM of DPPH, 100 µl of the plant extract solution was added. The mixture was shaken and allowed to stand at 20°C in dark for 40 minutes. After the decrease in absorbance, the resulting solution was monitored at 517 nm. The DPPH radical scavenging activity of phenolic compounds was expressed as mg/100g of dry matter of VCE in 40 minutes. The control solution was consisted by 100 µl of methanol and 2.90 ml of DPPH solution. The radical solution was prepared daily.

ABTS radical scavenging test: The method developed by Djeridane, et al. (2006) slightly modified was used in this experiment; 1.0 mM of AAPH solution was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution 100 mM potassium phosphate buffered (pH 7.4) containing 150 mM NaCl. The mixture was heated in a water bath at 68 °C for 20 minutes. The concentration of the resulting blue-green $ABTS^{\bullet+}$ (radical cation solution) was adjusted to an absorbance of 0.65 ± 0.02 at 734 nm. The sample solution of 60 µl was added to 2.94 ml of the

resulting blue-green ABTS radical solution. The mixture, protected from light, was incubated in a water bath at 37 °C for 20 minutes. Then the decrease of absorbance at 734 nm was measured. The control solution was consisted by 60µl of methanol and 2.94 ml of $ABTS^{\bullet+}$ solution. The stable ABTS radical scavenging activity of the phenolic compounds in the extract was expressed as mg/100 gm dry plants part powders of VCE. All radical stock solutions were prepared fresh daily.

Determination of antimicrobial activities

The following bacterial strains were employed in the screening: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and fungi: *Aspergillus niger, Candida albicans* given by IBiSE (UIT Thionville - Metz University). The antimicrobial activities were examined by the disk - diffusion method (Bauer, et al., 1966). The bacterial cell suspension was prepared from 24h culture and adjusted to an inoculation of 1×10^6 colony forming units per ml. Sterile nutrient agar (Immun präparate, Berlin, D, 26 g agar/ l distilled water) was inoculated with bacterial cells (100 µl of bacterial cell suspension in 25 ml medium) and poured into dishes to give a solid plate. Yeasts and hyphomycetes (1×10^6 colony forming units per ml) were inoculate into sterile Mueller-Hinton-agar (Becton Dickinson, Heidelberg) according to DIN E 58940-3 for the agar disc-diffusion assay (Al-Fatimi, et al., 2007).

10 µl of test material (250 gm/ 500 ml) dissolved in the same solvent like for extraction, were applied on sterile paper discs (6 mm diameter). Ampicillin, gentamicin, and nystatin were used as positive control, and the solvents water and methanol-water (50/ 50 v/v) as negative control. The solvents were allowed to evaporate in a stream of air and the discs were deposited on the surface of inoculated agar plates. Plates were kept for 1 h in refrigerator to permit good prediffusion of substances into the agar. The Plates with bacteria were incubated for 24 h at 37°C, the plates with yeast for 48h at 36 °C and the plates with hyphomycetes for 72 h at 30°C. Inhibition zone diameters around each of the disc (diameter of inhibition zone plus diameter of the disc) were measured and of the incubation time. An average zone of inhibition was calculated for the three replicates.

Minimal inhibitory concentrations (Mics) were determined by the agar diffusion technique as described by Rajbhandari and Schöpke (1999). The highest concentration of extract tested during the experiment was 2 mg/ ml. The MIC (minimum inhibitory concentration) corresponds to the lowest concentration of the tested extracts (water, or methanol - water extract), able to inhibit any visible microbial growth. Several concentrations of the extracts were prepared, (2.5, 2.0, 1.5, 1.0, 0.5, and 0.05 ml/ 10 ml) the different solutions were agitated vigorously. And approximately 20 µl ml of each concentration mixture was transferred in the disk. Then the disks were transferred in the Petri dishes containing the microorganism testing. The plates were incubated for 24 h at 37°C for bacteria, for 48 h at 36 °C for yeast, and for 72 h at 30 °C for the fungal. After incubation, the number of colonies in each plate was counted. Each assay replicated three times.

Anti-inflammatory activity by nitrite assay

The test used to assess the potential anti-inflammatory activity of molecules consisted of evaluating their capacity to inhibit NO production in activated macrophages (Pacheco-Sanchez, et al., 2007). Released nitrite (NO_2^-) in the culture medium was measured as an indicator of NO production according to the colorimetric test based on the Griess reaction. Briefly, 1ml of plant extract was mixed with 1ml of Griess reagent at room temperature for 30 min. The nitrite concentration was determined by measuring the absorbance at 548 nm using a standard curve of $NaNO_2$.

The results were expressed as percentage of NO production compared to the control as follows:

$$\% \text{ Inhibition} = 100 \times [NO_2^-] \text{ control} - [NO_2^-]_{Ex.} / [NO_2^-] \text{ control}$$

- With [NO2-] control is the concentration of nitrite released without addition of the extract, and
- $[NO_2^-]_{Ex}$ the concentration of nitrite released by the cells in presence of the plant extract.

***Statistical analysis*:** Results are presented as mean ± standard Error; statistical analysis of experimental result was based on analysis of variance one way ANOVA. Significant difference was statistically considered at the level of $P < 0.001$.

RESULTS

Chemical identified in Essential Oils: Yields of essential oils from the leaves of *F. capensis* were 0.65 ± 0.02 g/100 gm of dry leaves (w/w). The value is the mean of standard deviation (n = 3) ($P \leq 0.05$). Quantitative and qualitative analytical results are shown in Table 1.

Total phenolic contents: In table 2 is shown the amount of phenolic compounds analysis. The values are the mean ± SD (n = 3, w/w)

Qualitative and quantitative analysis of poplyphenolic compounds: The figures 1 and 2 show the corresponding RP-HPLC chromatogram of MWE and WE respectively. Peaks were identified and quantified on the basis of their retention time values and UV spectra by comparison with those of the single compound in the standard solution. The retention time and the concentration of polyphenolic compounds contained in the extracts are reported in the Table 3. There were numerous peaks that were not identified because of lack of suitable standards. The samples were analyzed at least four replications at 280 and 320 nm.

Antioxidant analysis: In table 4 is reported the DPPH and ABTS antioxidant test values.

Antimicrobial analysis: The results of antimicrobial tests are shown in tables 5 and 6.

Anti-inflammation properties of phenolic compounds: The anti-inflammation properties of EO, WE and MWE are reported in figure 3.

DISCUSSION

About 31 compounds are identified in the *F. capensis* essentials oil, as the results demonstrate the major are carvacrol (65.78%), α-caryophyllene (29.81), caryophyllene oxide (25.70 %), linalool (3.97%), 3 - tetradecanone (2.90 %); geranylacetone (1.20), 3,7, 11 - trimethyl-3-hydroxy-6;10-dodecadiene-1-yl acetate (1.53 %), hexahydrofarnesyl acetone (1.21 %), α- caryophyllene (0.81 %), 2- methyl-3-hexyne (0.69 %) and scytalone (0.69%). Most of the compounds identify play important roles in the aromas of essential oils for example, caryophyllene possess a woody-spicy odour with a somewhat bitter taste and has been used particularly for chewing gum as well as in spice blends and flavour compositions (El-Massy, et al., 2009). But, several authors showed that the relative composition of some compounds change significantly because of the use of dry leaves (Omidbaigi, et al., 2004). Volatile aroma compounds are the most sensitive components in the process of food drying but the loss of volatiles in herbs and spices during drying depends mainly on drying conditions and the biological characteristics of plants; the antioxidant activity of essential oils was also affected (Omidbaigi, et al., 2003). The results of phenolic compounds content reveal that the leaves of *Ficus capensis* are mainly composed by total polyphenolic compounds (16.47 mg GAE/g dw). The amounts of total

anthocyanin and total flavonoid are 9.52 mg CgE/g dw and 6.92 mg CE/g dw respectively. The amount of total tannins observed was weak (4.21 mg CE/g dw). Quantitative and qualitative analysis of individual phenolics compounds by HPLC show that water is not the best extract solvent for phenolic compounds. This is in concordance with Muanda, et al., (2009), which report that an aqueous alcohol is considered to be the best solvent for extracting phenolic compounds from plant materials.

These results show the most important compound identifies in WE is epicatechin (1.21 mg/ml) and the less one is catechin (0.19 mg/ml). While in MWE, rutin (1.69 mg/ml) is the major. For the antioxidant property, it is well known that plants contain various antioxidants such as ascorbic acid, tocopherols, polyphenols and terpenoids (Graddmann, 2005). Phenolic compounds have received much attention as one of the principle antioxidants found in plants; several authors reported that some essential oils and organic solvent extracts from plants possess antioxidant activity (El-ghorab, et al., 2007).

In this study, the radical scavenging effect of leaves extract was determined by ABTS and DPPH colouring methods and the values were comparing to VCE. The radical scavenging effects of the E.O (Essential oils), MWE (Methanol-water extract) and WE (Water extract) extracts reveal a positive control on both ABTS and DPPH tests (Table 3). The ABTS test and the DPPH values express as mg VCE / g dw varied between 1.37 and 0.09 mg. In addition to compare this antioxidant capacity with the others plants previously described in the literature such as *Mangosteen* fruit, *Melissa officinalis*, *Matricaria recutita*, *Matricaria recutiat*, *Cymbopogon citrate*, the antioxidant activity was expressed as IC_{50}. The IC_{50} values of *F. capensis* leaves extracts (MWE, WE, E.O) by DPPH assay were $IC_{50:}$ 10.25, 13.07 and 23.34 µg/ml respectively (Table 3). These results, compared to the extract from *Mangosteen* fruit (IC_{50}: 10.94 µg/ml) (Pothitirat, et al., 2009), *Melissa officinalis* (IC_{50}: 32. µg/ml (WE), (IC_{50}: 24.3 (ME) µg/ml), *Matricaria recutita* (IC_{50}: 947.2 µg/ml (WE), (IC_{50}: 115.9 µg/ml (ME) and *Cymbopogon citrates* IC_{50}: 1615.7 µg/ml (WE), (IC_{50}: 85.7 (ME) µg/ml (Pereira, et al., 2009) reveals that *F. capensis* leaves extract has significant antioxidant activities.

Phytochemical screening of leaves extracts of *F. capensis* revealed the presence phenolic compounds (polyphenols, flavonoids, anthocyanins, tannins, terpenoids, etc.), the presence of these metabolites reveals its activity against pathogenic bacteria. According to Ebana, et al. (1991), alkaloids inhibit pathogenic bacteria and tannins are important in herbal medicine in treating wounds and to arrests bleedings (Nguyi, 1988). This also confirms the use of *F. capensis* for wounds in circumcision (Oyeleke, et al., 2008).

The extracts had varying degree of antimicrobial activity against the test organisms. The MWE has the highest activity with zone of inhibition between 10 – 21 mm as compared to that of the WE. The essential oil showed moderate activity (Table 4). This antioxidant activity may be due to the presence of terpenes with antioxidant activity such as, caryophylene, linalool and carvacrol with strong antioxidant activity (El-Massy, et al., 2009). The results are in accordance with those found by Oyeleke, et al. (2008). The minimum inhibitory concentration (MIC) obtained reveals the effectiveness of the plant extract as chemotherapeutic agents. The organisms were inhibited at concentrations > 1000 µg/ml (Table 5). The most susceptible organisms to the antimicrobial activity of *F. capensis* were E. coli and *Bacillus subtillis*.

About the anti-inflammation properties; the anti-inflammatory activity of EO, WE and MWE was studied for their inhibitory effects on chemical mediators release

(LPS/IFNc-induced NO production) from macrophages. Activated macrophages produce large amounts of chemical mediators that indicate inflammation. NO, a bioactive free radical, is one of these critical mediators which is produced by inducible NO synthase (iNOS) in inflammatory macrophages when stimulated with LPS/ IFNc mixture (Pacheco-Sanchez, et al., 2007). Excessive production of NO is indicated both in chronic and acute inflammation (Terra et al., 2007). In fact, NO production induced by LPS/ IFNc through iNOS induction may reflect the degree of inflammation and may provide a measure for assessing the effect of the extract/ fractions on the inflammatory process. As shown in Figure 4, the addition of EO at different concentrations (20, 50, 100 and 150 µl) significantly reduced NO production , resulting in 15.05%, 22.03%, and 39.80% inhibition of NO production in macrophages stimulated with LPS/IFNc, respectively. As for the WE and the MWE, their use showed significant inhibitor effect varying between (22.03 to 48.08 %) for WE and (36.75 to 75.98 %) for MWE. These results show a significantly reduced NO production in a dose-dependent manner. Similarly to theses results, it has been demonstrated in several studies that extract rich in flavonoids and proanthocyanidins inhibited NO production in RAW 264.7 macrophages (Clancy, et al., 1998; Ho, et al., 2007; Diouf, et al., 2009). These results seem to indicate that phenolic compounds present in the extracts are responsible for the anti-inflammatory activity. Nevertheless, in the EO, the anti-inflammatory activity is correlates positively with the radical - scavenging activity and the highest antioxidant activity was correlated with the highest total phenol content and/or the highest anti-inflammatory activity of the tested extract/fraction.

In conclusion, the phytochemicals, the antioxidant, anti-inflammation and antimicrobial activities of the leaves of *Ficus capensis* were evaluated; the results demonstrate that the MWE was more effective against pathogens and the inhibition percentage of NO was high. These properties are correlated to the antioxidant properties. The results of the present study support the view that the leaves of *F. capensis* could be a potential source of natural antioxidant/antimicrobial and anti-inflammation drugs.

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Table-1: Main components (%) of the essential oils of *Ficus capensis* (leaves).

Num.	Rt (min)	Compound	Concentration (%)
1	3.04	3-hexen-1-ol	0.67
2	3.09	2- pentyl- Furan	0.40
3	3.13	Linalool	3.97
4	3.15	pelargonaldehyde	0.27
5	3.17	caprinaldehyde	0.21
6	3.19	2-decenal	0.28
7	3.33	theaspirane	0.41
8	6.95	2-tert- buty-3,4,5,6-tetrahydropyridine	0.34
9	7.07	capric acid	0.18
10	13.40	terpinolene	0.25
11	13.50	geranylacetone	1.20
12	13.94	α- caryophyllene	29.81
13	18.22	ß-ionone	0.38
14	20.09	3,7,11-trimethyl-3-hydroxy-6,10-dodecadiene-1-yl acetate	1.53
15.	20.26	caryophyllene oxide	25.70
16	20.88	3-tetradecanone	2.90
17	20.96	2-methyl-3-hexyne,	0.69
18	21.70	3,3- dimetyl - hepatne	0.23
19	21.84	myristaldehyde	0.46
20	22.07	myristic acid	0.16
21	22.08	5,10-pentadecadien-1-ol,(z,z)-	0.17
22	23.43	hexahydrofarnesyl acetone	5.21
23	24.07	scytalone	0.69
24	29.08	1-hexyl-2-nitrocyclohexane	0.65
25	27.93	carvacrol	65.78
26	50.96	cetylic acid (Palmitic Acid)	0.19
27	51.07	*2*-methyloxazoline	0.19
28	51.15	isopropenyl methyl ketone	0.22
29	51.49	2-methyl-4-oxo-2 pentenoic acid	0.26
30	51.68	3-butenyl propyl Ether	0.27
31	51.81	heptacosane	0.20

Table-2: *Ficus capensis* leaves phenolic compounds composition.

Extract mg/gm dw	TPC (GAE)	TFC (CE)	TAC (CgE)	CT (CE)
MWE (50/50 v/v)	16.47	6.92	9.52	4.21

Table- 3: MWE and WE compounds identify in *F.capensis* leaves and their concentrations by HPLC.

Name of compound	Rt (min)	MWE (mg/ml)	WE (mg/ml)
gallic acid	11.98 ± 0.22	0.28 ± 0.05	n.d
protocatechuic acid	14.78 ± 0.92	0.37 ± 0.06	0.45 ± 0.01
catechin	23.96 ± 0.23	0.63 ± 0.02	0.19 ± 0.01
caffeic acid	28.92 ± 0.29	0.82 ± 0.09	n.d
epicatechin	31.45 ± 0.55	0.24 ± 0.03	0.28 ± 0.02
p-coumaric acid	33.25 ± 0.36	0.85 ± 0.11	1.21 ± 0.04
rutin	37.02 ± 0.36	1.68 ± 0.13	0.17 ± 0.02
quercetin glucosyl	37.71 ± 0.18	0.98 ± 0.08	0.29 ± 0.03
quercetin dihydrat	39.47 ± 0.19	n.d	0.25 ± 0.02
cinnamic acid	41.68 ± 0.65	0.83 ± 0.06	0.20 ± 0.01
quercetin	42.43 ± 0.30	0.19 ± 0.04	0.46 0.02

- WE: water extract; MWE: Methanol water extract; Rt: retention time in minute, nd: not detected,
- values are mean ± SD of three determinations.

Table-4: AOA of *F. capensis* leaves extract.

Extracts	ABTS	DPPH		
	mg VCE/gm dw	mg VCE/gm dw	% IP	Ic_{50} (µg/ ml)
E.0	0.14 ± 0.03	0.09 ± 0.01	56.83	23.34 ± 0.07
MWE	1.37 ± 0.22	0.98 ± 0.02	83.32	10.25 ± 0.03
WE	0.60 ± 0.05	0.43 ± 0.01	71.89	13.07 ± 0.02

- Values are mean ± SD of three determinations.

Table-5: Antibacterial activity of leaf and stem bark extracts of *Ficus capensis*.

	Organisms (zones of inhibition in mm)					
Leaf extracts	E.c.	C.a.	A.n.	P.a.	B.s.	S.a.
MWE	21	10	24.3	n.a	16	10.0
WE	8	n.a	n.a	n.a	11.0	n.a
Wdd(-)	0	0	0	0	0	0
MW(-)	0	0	0	0	0	0
Ap.(+)	58 ± 3	n.a	n.a	45 ± 3	55 ± 3	56 ± 3
Gt.(+)	55 ± 2	n.a	n.a	45 ± 2	45 ± 2	44 ± 2
Ny(+)	n.a	53 ± 1	47 ±1	48 ±1	47 ±1	47 ±1

- Values are mean ± SD of three determinations
- S.a.: *Staphylococcus aureus*; B.s.: *Bacillus subtillis*; E.c.: *Escherichia coli*; P.a.; *Pseudomonas aeruginosa*; A.n.; *Aspergillus niger*; C.a.: *Candida albicans*; WE: water extract; MWE: methanol-water extract; W(-): didistillate water negative control, MW (-): methanol - water (50/50 v/v) negative control ; Ap(+): Ampicillin positif control; Gt(+): gentamicin positif control; Ny (+): Nystanin positif control; Na: not actif.

Table 6: Minimal inhibitory concentrations of E.O, MWE and WE.

	MIC (µg/ ml)					
Samples extract	E.c.	C.a.	A.n.	P.a.	B.s.	S.a.
E.0	> 1500	NI	NI	NI	> 1500	> 1500
MWE	> 500	> 1000	>1000	NI	> 500	> 500
WE	> 1000	NI	NI	NI	> 1000	NI

- NI: not inhibited; values are mean ± SD of three determinations

Figure Captions

Figure-1a: chromatogram *Ficus capensis* leaves extract (MWE) 280 nm.
1: gallic acid, 2: protocatechuic acid, 3: catechin, 4: caffeic acid, 5: epicatechin, 6: *p*-coumaric acid, 7: rutin, 10: cinnamic acid, 11: quercetin

Figure-1b: chromatogram Ficus capensis leaves extract (MWE) 320 nm.
1: gallic acid, 2: protocatechuic acid, 4: caffeic acid, 5: epicatechin,
6: p-coumaric acid, 8: quercetin glucosyl, 10: cinnamic acid, 11: quercetin

Figure-2a: chromatogram WE F. capensis leaves analysis (280nm).
5: epicatechin, 6: p - coumaric acid, 8: quercetin glucosyl, 9: quercetin dihydrat, 10: cinnamic acid, 11: quercetin

Figure-2b: WE chromatogram F.capensis (320 nm).
2: protocatechiuc acid, 3: catechin, 5: epicatechin, 6: p-coumaric acid, 7: rutin, 8: quercetin glucosyl, 9: quercetin dihydrat, 10: cinnamic acid

Figure-3: Relationship between inhibition percentage of NO_2^- and the concentration (volume) of $[NO_2]^-$.
IP: % Inhibition